binding energy. Together, the simulations and experimental results suggest that PI-PLC binding to PC-rich membranes is mediated by aromatic-rich surface-accessible regions of the protein that engage in a myriad of transient pi-cation interactions with choline headgroups. This PC-binding mechanism may be utilized by other peripheral membrane proteins.

398-Pos Board B184

Hydrophobic Matching Between Phospholipid Bilayers and Helical Peptides Affects the Memrbane Affinity of Sterols

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The lateral organization of cell membranes is important for several cellular functions including signaling and membrane trafficking. In addition pathogen uptake and influenza virus budding is associated with sphingolipid and cholesterol enriched lateral membrane domains. The lateral structure of membranes is in part controlled by lipid self-organization, but it is becoming increasingly clear that also membrane proteins can play an active role in the maintenance of the lateral structure. Cholesterol is thought to have an important role in lateral organization of eukaryotic cell membranes. As cholesterol also has been implicated to take part in the sorting of cellular transmembrane proteins it is a good starting point to determine how transmembrane proteins influence the lateral sorting of cholesterol in phospholipid bilayers. Insight into this can be obtained by studying how cholesterol interacts with bilayer membranes of different composition in the presence of different transmembrane peptides, mimicking the transmembrane helices of proteins. By measuring the equilibrium partitioning of the fluorescent cholesterol analogue cholestatrienol (CTL) between large unilamellar vesicles and methyl-beta-cyclodextrin the effect of hydrophobic matching on the affinity of sterols for phospholipid bilayers was determined. The results showed that increasing positive mismatch led to higher affinity of the sterol for the bilayers. This suggests that hydrophobic matching could affect the lateral organization of cholesterol in cell membranes and have an important role especially in membrane trafficking.

399-Pos Board B185

A Flim-FRET Study of the Raft-Associated Proteins in Erythrocytes Expressing the Miltenberger Blood Group Antigen Subtype III (Mi.III) Phenotype

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Lipid rafts are dynamic signaling hubs packed with ordered lipids on the cell membrane. Lipid rafts in erythrocytes can be classified into two types: flotillin-associated rafts and stomatin-associated rafts. We previously found by biochemical means that these two types of rafts coalesce on the erythrocytes expressing the Mi.III blood type. Mi.III is one of the most important blood groups in the field of transfusion medicine in Southeast Asia. Mi.III encompasses a characteristic hybrid structure of glycophorin A and glycophorin B, termed Gp.Mur, and is part of the band 3 macrocomplexes on the erythrocyte surface. Band 3 is known to be partially associated with erythrocyte rafts. The objective of this study was to examine if Gp.Mur was associated with rafts on Mi.III+ cells. We assessed the protein-protein interaction between Gp.Mur and band 3 by measurements of FRET using fluorescent lifetime imaging microscopy (FLIM). In these experiments, band 3 and GFP-Gp.Mur fusion constructs were co-transfected into HEK-293 cells, and band 3 was labeled with Alexa Fluor-568 prior to FLIM-FRET measurements. Here, GFP served as the donor and Alexa Fluor-568 as the acceptor fluorophore if FRET occurred. We found that the fluorescence lifetime of the donor was significantly shorter at some surface edges in the cells coexpressing Gp.Mur-GFP and band 3; this phenomenon however was not observed in the cells expressing Gp.Mur-GFP only. We thus conclude that Gp.Mur and band 3 interact within 10 nm on particular regions of the plasma membrane, some of which are likely lipid rafts. Because Mi.III+ erythrocytes are superior in CO_2 metabolism, pH homeostasis and cell membrane resilience, as compared to the non-Mi.III cells, their unique raft organization is expected to have functional implications.

400-Pos Board B186

Membrane Interaction of α-Synuclein in Different Aggregation States

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Aggregated α-synuclein in Lewy bodies and Lewy neurites are hallmarks of Parkinson's disease (PD). Recent observations that aggregated α-synuclein is propagated to healthy neurons grafted into brains of PD patients prompted our study. We asked whether any, and if so which, molecular form(s) of α-synuclein can pass over model phospholipid bilayers. Confocal fluorescence microscopy was used to study the binding of Alexa488 labeled α-synuclein to giant unilamellar vesicles (GUVs) and to monitor if the α-synuclein is transported over the phospholipid bilayers. The GUVs were composed of mixtures of DOPC and DOPS or DOPC and cardiolipin at different molar ratios to vary the membrane charge. All lipids chosen for this study are common in human membranes. Cardiolipin is primarily found in the mitochondrion, i.e. an organelle that is implicated in PD pathogenesis.
We studied membrane binding and transport for monomeric, fibrillar as well as on-pathway α-synuclein samples. To enable preparation of samples representing defined time points during the aggregation lag period, conditions were identified that resulted in reproducible aggregation kinetics at moderate shaking and at quiescent conditions. We observed association to lipid membranes for on-pathway species and equilibrium aggregates, but not for monomers. Particularly strong association was found between α-synuclein and GUVs that contained cardiolipin or DOPS. By contrast, α-synuclein did not associate with GUVs containing DOPC only. No transport of α-synuclein over the GUV bilayer was observed under any of the conditions studied. Labeled α-synuclein was never observed inside the GUVs although clearly visible in the surrounding buffer. This suggests that the transport of α-synuclein over membranes, which has been observed in several previously published cell culture experiments, requires additional molecular components and/or an active transport mechanism.

401-Pos Board B187

Influence of the Membrane Composition of Retinal Photoreceptors on the Reversible Binding of a Neuronal Calcium Sensor Protein (NCS), Recoverin : A Solid-State NMR and FTIR Study

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NCS proteins consist of 4 domains called EF-Hand, which are 2 α-helices joined by a loop with a highly conserved sequence. Binding of calcium ions by this loop induces important conformational changes in the protein. Most of the NCS also have a N-terminal sequence recognized by N-myristoyl transferase which is responsible for the acylation of the proteins. At low calcium concentration, the myristoyl group is sequestered into a hydrophobic cavity. The binding of 1 to 4 Ca^{2+} leads to the extrusion of the myristoyl (known as the calcium myristoyl switch) and the exposure of many hydrophobic residues allowing the protein to go from a cytosolic form to a membrane bound form. This property has an important biological function in the visual phototransduction cascade. In fact, the absorption of a photon by the visual pigment rhodopsin leads to an important decrease of calcium level in photoreceptors and recoverin, one of the NCS family, plays a key role in the recovery phase of visual excitation by inhibiting rhodopsin kinase at high calcium level. An interesting fact is that the membrane composition of the photoreceptor rod outer segments is known to be very different from that of other membranes, with more than 60% of the lipids being polyinsaturated. We have determined in the present study how the membrane composition affects the reversible membrane binding of recoverin. More specifically, ^{31}P solid-state NMR and ^{2}H solid-state NMR have been used to get information respectively about the lipid polar head groups and the recoverin myristoyl group. FTIR was also used to study the structure of recoverin (amide I' band) and the lipid acyl chains (CH_2 symmetric stretch band).

402-Pos Board B188

Identifying Motifs for Phosphatidylcholine Activation of Bacterial Phosphatidylinositol-Specific Phospholipase C Enzymes

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Phosphatidylinositol-specific phospholipase Cs (PI-PLCs) secreted by pathogenic bacteria are often virulence factors. For one well-studied example, *Bacillus thuringiensis* PI-PLC (B*t*PI-PLC), the presence of the non-substrate lipid phosphatidylcholine (PC) in membranes enhances both membrane binding and enzymatic activity towards phosphatidylinositol (PI). A strip of four surface-exposed Tyr residues near the rim of the α/β-barrel are involved in this enhancement, and it has been proposed that this strip contributes to a specific PC binding site. The related PI-PLC from *Staphylococcus aureus* (S*a*PI-PLC), has similar kinetic characteristics to B*t*PI-PLC, but has only two Tyr residues in the same region. While S*a*PI-PLC can also be specifically activated towards PI cleavage by incorporation of PC into assay systems, S*a*PI-PLC membrane binding as a function of PC content is considerably weaker than that of B*t*PI-PLC. Mutagenesis of the two S*a*PI-PLC Tyr residues (Y253S/Y255S) reduces interfacial activity of S*a*PI-PLC. Adding the two 'missing' Tyr residues (N254Y/H258Y) did not further enhance S*a*PI-PLC specific activity but it dramatically enhanced binding of the protein to PC-rich

vesicles. This suggests that PC activation is more complicated than simply binding the protein to membranes. Comparing enzyme kinetics and the effects of mutations on B*t*PI-PLC, S*a*PI-PLC and the PI-PLC from an intracellular pathogen (*Listeria monocytogenes*) suggests a rationale for the acquisition of a discrete PC binding site in some, but not all, PI-PLC enzymes.

403-Pos Board B189

Role of Surface Modifications in Ovalbumin Interacting with Lipid Membranes

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Recent electrophysiology studies in our laboratory suggest that some proteins from the Serine Protease Inhibitor (SERPIN) family cross cell membranes without regulated uptake mechanisms. Since these proteins do not structurally resemble typical membrane proteins, questions arise about the factors governing interactions between lipid and protein of this family. Previous studies of ovalbumin_a chicken protein of the SERPIN family_indicate that both glycosylation and phosphorylation play vital regulatory roles. Combining UV-visible and phosphorus NMR spectroscopy offers information on these posttranslational modifications. Proteins crossing the cell membrane must enter the central region of lipid tails. This hydrophobic environment causes the proteins to change conformation and altering the absorbance and resonance spectra. In UV-spectroscopy, environmental hydrophobicity cause phenylalanine, tyrosine, and tryptophan peaks to shift. In P31 NMR, the hydrophobic interactions lead to changes in phosphorus chemical shifts. Combining information from both these methods reveals the interactions of phosphorylated glycoproteins and lipid bilayers.

404-Pos Board B190

Measuring Initial Insertion Forces of Ion Channels: The Impact of the Alpha-Hemolysin Phosphocholine Binding Pocket

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Alpha hemolysin (αHL) from *Staphyloccocus aureus,* spontaneously forms channels in lipid bilayers through a process of monomer aggregation and bilayer translocation. However, little is known about the forces required to initially part the outer lipid leaflet. Crystallographic studies have revealed the location of a phosphocholine binding pocket at the base of αHL's extracellular cap. As a selective gatekeeper of lipid/protein interaction, this pocket plays a putative role in the pore formation process.

Here, we explore lateral surface pressure changes in the initial stages of channel formation using a Langmuir monolayer trough. Lipid monolayers limit the process to its initial stages by prohibiting full bilayer translocation. Surface pressure results are compared to whole cell lysis experiments, where red blood cells (RBC) are used to measure the protein's ability to form fully functioning pores.

Two αHL mutations, R200A and W179R, were expressed and purified along with wild type (WT) to compare channel behavior in RBCs and in the presence of lipids containing phosphocholine (PC) or glycerol phosphate (GP). These mutations were strategically chosen to systematically alter the structure and charge interactions with headgroups in the binding pocket.

We find that W179R dramatically reduces RBC lysis. Monolayer measurements with either PC or GP show elevated or suppressed lateral surface pressures relative to WT, depending on the headgroup. In addition, the WT shows enhanced lateral surface pressures in GP lipid. These data indicate that the WT binding pocket plays a lipid-dependent role in membrane interaction. They also suggest that an anticorrelation exists between lytic ability and the monolayer area expansion. In this view, the WT creates a stable pocket around a phosphocholine head group, allowing pore formation to proceed efficiently, without expending energy unnecessarily on the separation of lipids.

405-Pos Board B191

Interaction of an Acidic Peripheral Protein with Anionic Lipid Membranes: Insights from Molecular Dynamics

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The fatty acid-binding protein ReP1 is a soluble protein that shows the common beta-barrel motif with two alpha-helix in the portal region. Its isoelectric point of 5.85 leads to net charge of −1 at neutral pH. Previous evidence of its interaction with anionic lipids [1], makes it an attractive model to test the hypothesis that the binding and orientation of soluble proteins within lipid membranes are driven by the interaction of its macrodipole with the interphasial electric field [2].

The interaction of ReP1 with lipid membranes of anionic and zwitterionic phospholipids was studied by multiple-run molecular dynamics, Potential of Mean Force calculations and filtration assays. ReP1 has a macrodipole of 310 Debyes pointing towards the portal region. We found that it interacted selectively with anionic interphases, aligning its macrodipole in the configuration of lowest energy within the membrane electric field. Additional evidence of this orientation was achieved experimentally by FRET measurements. This orientation led to the portal region in contact with the membrane. The strength and range of the interaction and the preference in spacial configuration was attenuated by the presence of salt. A global loss of compactness was seen by MD and FT-IR spectroscopy.

A similar behaviour has been described for L-BABP [2], a protein with identical tertiary structure.

These electrostatic-like features of the interaction suggest that the interphasial electric field could be the driving force for the binding and orientation, and may be involved on the ligand delivery mechanism in this family of proteins.

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406-Pos Board B192

Effect of Hydrophobic Peptide Sequence upon Peptide-Dependent Acceleration of Lipid Flip-Flop

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The asymmetric distribution of different lipid species in the inner and outer half of the bilayer is an important feature of many natural membranes. Certain transmembrane (TM) helix-forming peptides are known to increase the transverse diffusion (flip-flop) of lipids and can break down lipid asymmetry. We have investigated the dependence of peptide enhanced flip-flop upon TM helix sequence in order to try to understand the mechanism by which this occurs.

The movement of a fluorescent phosphatidylcholine analog (6-NBD-PC) from the outer to inner leaflet was used to measure the influence of different model TM helices on the natural flip-flop rate of model membranes. The most hydrophobic TM peptides had very little or no effect upon flip-flop rate. Preliminary results indicate that for TM peptides of comparable length, a reduction of hydrophobicity increases the lipid flip-flop rate. The lipid flip-flop rate was most markedly increased when an Asp residue was introduced near the center of a highly hydrophobic sequence. The flip-flop was greatly accelerated when the Asp residue was protonated and the peptide was in a TM configuration but accelerated the most when the Asp residue was deprotonated and the peptide resided on the bilayer surface. Although certain pore forming peptides have been shown to accelerate flip-flop, no pore formation was detected for the peptides used in our studies. We conclude that perturbation of lipid packing is a possible mechanism by which hydrophobic helices accelerate flip-flop, while pore formation is unlikely to be a universal mechanism for accelerating flip-flop.

407-Pos Board B193

Amyloid-Beta 1-42 Binds Preferentially to Nanoscale Electrostatic Domains in Cholesterol-Enriched DOPC Lipid Membrane

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The plasma membrane is a complex structure, composed primarily of phospholipids and other macromolecules, such as proteins, sterols and steroids. It plays a vital role in cell motility and acts as a barrier for extracellular materials. Amyloid fibrils are linked to multiple neurodegenerative diseases, such as Alzheimer's. Although fibril plaque formation is associated with biological membranes *in vivo*, the role of the lipid membrane in fibril formation and toxicity is not well understood. We investigated the interaction of model lipid membranes with Aβ 1-42 peptide. Using Atomic force microscopy, we demonstrated that binding of Aβ 1-42 peptide to DOPC bilayers with 20% cholesterol is non-uniform and resulted in the formation of nanoscale islands loaded with A-beta. We attribute this effect to the presence of electrostatic nanoscale domains induced by cholesterol in the DOPC bilayer. These domains were resolved by AFM imaging of the lipid bilayer and by frequency modulated Kelvin probe force microscopy in the monolayer samples prepared with the Langmuir-Blodgett monolayer technique.